RESPONSES OF SPECIFIC NEUROSECRETORY CELLS OF THE COCKROACH, BLABERUS GIGANTEUS, TO DEHYDRATION [1]

BETTY J. WALL AND C. L. RALPH

Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pennsylvania

So much attention has been given to the regulation of water content in vertebrates (Sawyer, 1961) it is surprising that so little work has been done concerning the same processes in insects. The limited information that has accumulated about osmoregulation in insects is in part conflicting (see Discussion). Furthermore, as is generally true in all kinds of endocrine studies on insects, workers have confined their attention to the brain and corpora cardiaca-allata complex as possible sources of endocrines of water metabolism (Day, 1943; Stutinsky, 1953; Altmann, 1956; Raabe, 1959), in spite of the fact that the vast majority of neurosecretory cells in some insects are in the ventral ganglia (Geldiay, 1959; Füller, 1960).

This investigation was undertaken to observe by histological methods the entire complement of neurosecretory elements in the major ganglia of the cockroach, *Blaberus giganteus*, under normal and dehydrated conditions, to see if changes in certain ones could be detected, and thus perhaps implicate them in osmoregulation. Specific responses were observed in the neurosecretory cells of the pars intercerebralis and corpora cardiaca, which confirms the observations of others (Pflugfelder, 1937; Nayar, 1957), and in the type A cells in the three thoracic ganglia, an observation that has not been reported previously.

MATERIALS AND METHODS

The experiments utilized adult, male *Blaberus giganteus* L.[2] Prior to use the animals were maintained in mixed colonies of about 25 animals each in an animal room which was illuminated from 6 A.M. to 8 P.M. E.S.T. They were provided at all times with Purina lab chow and water. Male roaches were picked randomly from the colonies and placed individually in quart Mason jars with lids in which screen-wire discs were substituted in the retaining rings for the inserts. Four groups were set up:

Group	Food	Water
A	+	+
B	−	+
C	−	−
D	+	−

Groups A and B received a vial of water plugged with cotton. Groups A and D were given a pellet of lab chow.

[2] Cockroaches for establishing colonies were obtained from the Gulf Research and Development Company, Harmarville, Pennsylvania.
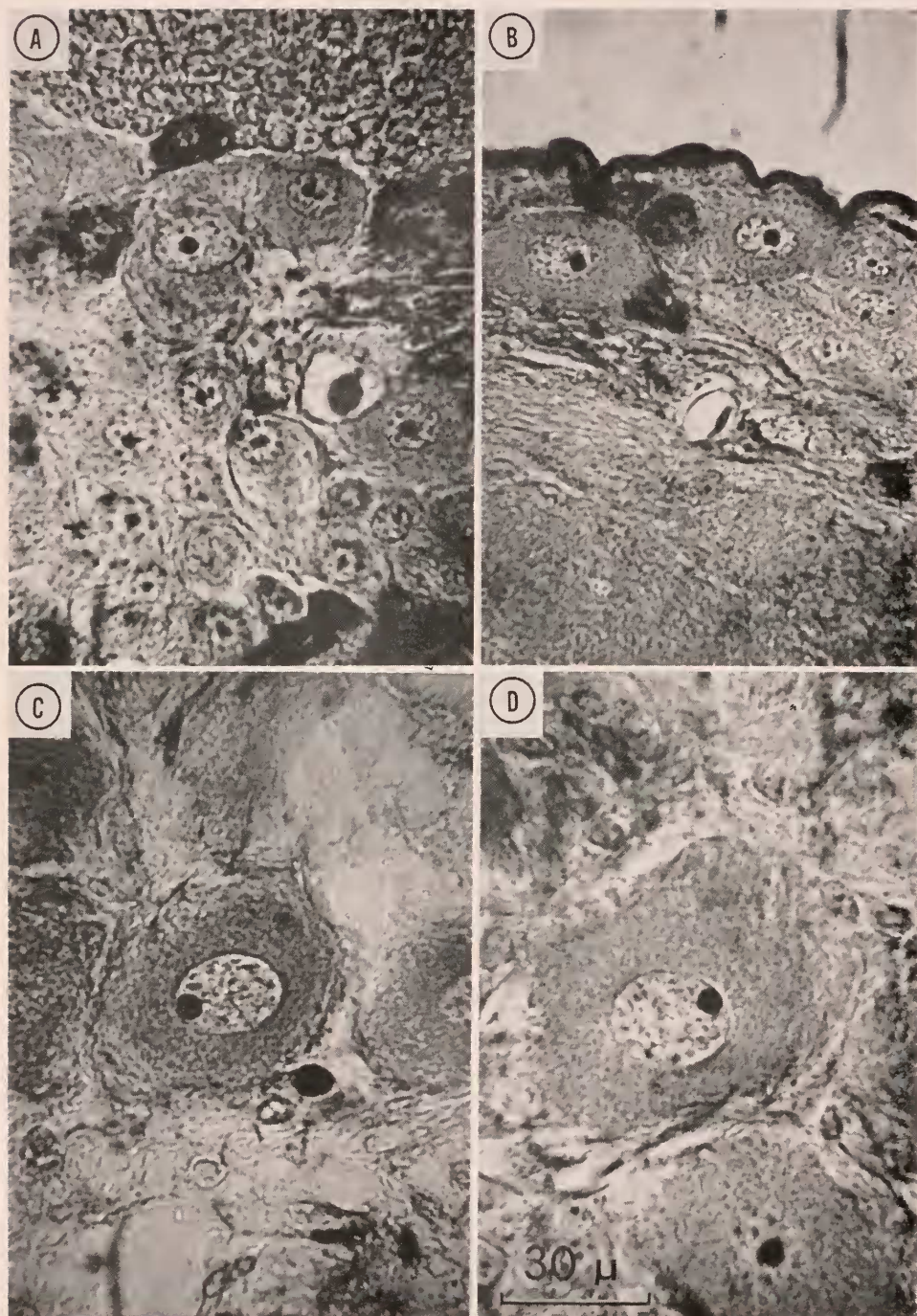
FIGURE 1.

An animal from each group was sacrificed at 4 P.M. on different days after the start of the experiment up to 12 days. All animals were sacrificed by an injection of Bouin's fixative (modified by Halmi, 1952) into the hemocoel and the nerve cord was carefully dissected out in saline solution (Hoyle, 1953). The tissue was fixed in Bouin's, washed, embedded, sectioned at 10 microns and then stained with either the aldehyde fuchsin (AF) of Gomori-Halmi-Dawson (Dawson, 1953) or Mallory's triple stain (Gray, 1954).

To compare the effects of NaCl injection with those of dehydration, other animals were injected with $4 \times$ NaCl in Hoyle's solution at 4 P.M. and then sacrificed after 24 hours. The nervous tissue was treated in the same manner as just described.

RESULTS

A total of 75 animals were used in seven replications of the basic experiment. In three experiments the cockroaches were maintained for 3, 5, 7, and 9 days under the experimental conditions. In one experiment animals were sacrificed at 5, 8, and 12 days, while in three other experiments they were sacrificed at 5 and 6, 5 and 7, and 5 and 8 days only. The results of all the experiments are in good agreement. (Although Mallory's triple stain was used in one experiment, all the observations are based on AF staining.)

The animals deprived of water showed loss of weight, 0.05–0.1 g. per day (about 1–2% of total body weight), while those given water did not change weight significantly. No differences could be detected between Groups A (with food) and B (without food), both of which were given water. Also, the effects of dehydration were the same for C and D (both without water), although Group C was not given food. Thus, it appears that nutritional states cannot account for the results observed in Groups C and D.

The effects of dehydration were quite pronounced after five days in both Groups C and D, producing specific effects on type A neurosecretory cells (Füller, 1960) of the pars intercerebralis and thoracic ganglia. Specific changes were not detected in any of the type A neurosecretory cells of the subesophageal or abdominal ganglia, or in any other neurosecretory cell types (Fig. 1). Periods of dehydration longer than 5 days, however, tended to produce non-specific alterations in the entire neural structure.

The smaller, or α-type, A cells of Füller (1960) (type I cells of Geldiay, 1959) in the pars intercerebralis of the supraesophageal ganglion showed specific cytological changes under dehydration. These cells in a dehydrated animal appear intensely and almost homogeneously purple after AF staining as compared to the purplish, more granular cytoplasm of the normal animal (Fig. 2). Also, the axons of the cells of the pars intercerebralis show a much greater amount of stainable substance in the fiber tracts, when compared to the appearance of comparable sections from control animals. In the corpora cardiaca of dehydrated animals there is a comparable accumulation of stainable material (Fig. 3).

FIGURE 1. Type B neurosecretory cells from those of normal and dehydrated animals. A, pars intercerebralis of normal animal (Group A); B, pars intercerebralis of dehydrated animal (Group D); C, thoracic ganglion of normal animal (Group A); D, thoracic ganglion of dehydrated animal (Group D). Cells from dehydrated animals do not differ from normal animals.
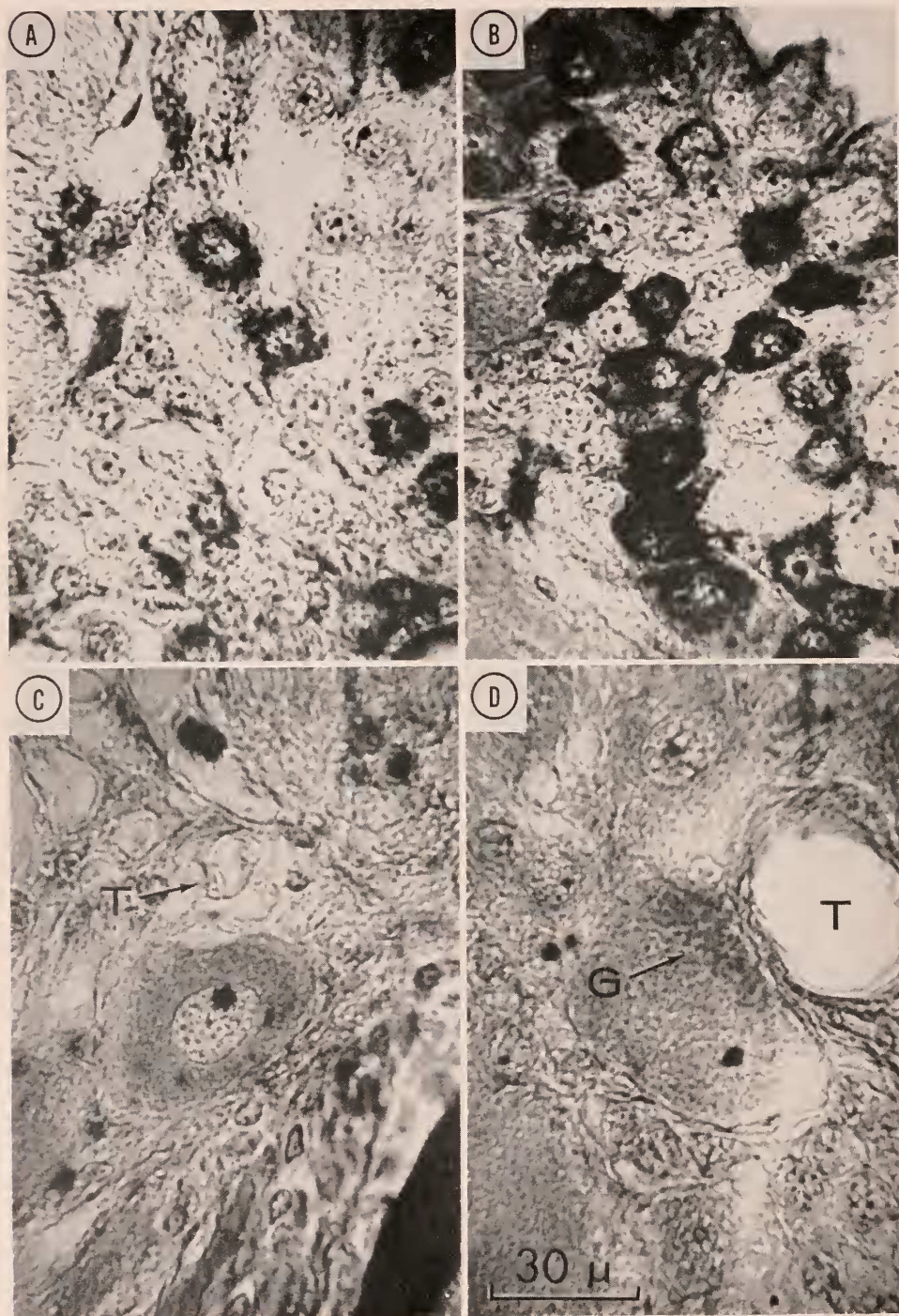
FIGURE 2.

The kind of A cell found in the thoracic ganglia, the larger, so-called β-type (Füller, 1960), is affected in quite a different manner. (There are about four of these cells in each ganglion.) In dehydrated animals their cytoplasm appears to be coagulated and relatively depleted of stainable granules, except for a dense mass of material clumped to one side of the cell. These cells always appear to be closely associated with a prominent trachea and the site of clumping is always toward the cell boundary adjacent to the trachea (Fig. 2).

No other cytological changes, such as nuclear or nucleolar size or morphological differences, could be detected in the A cells of either the pars intercerebralis or thoracic ganglia.

Discussion

It is assumed that the stainable granules are neurohormones or, more likely, the protein carriers of the hormones (Sloper, 1958). Accumulation of granular material by the type A cells of the pars intercerebralis may be variously interpreted. It could mean that the cells are sources of an antidiuretic factor, as evidenced by their great activity—granule production—when desiccated. On the other hand, a diuretic factor may be secreted which, to allow water conservation, is retained—hence, granule accumulation. The latter interpretation agrees with that of Nayar (1957) who forcibly fed *Iphita limbata* (Hemiptera) salt water and found that the pars intercerebralis neurosecretory cells were "loaded with stainable colloids" and "release of neurosecretory material to the blood appears to be inhibited." Also, animals under conditions that cause them to take up water are described as having "comparatively colloid-free neurosecretory cells."

However, Nayar in a later work (1960) gives another description of the neurosecretory cells of the same animal. Those subjected to salt-water feeding or injections are said to show a "characteristic clumped pattern" of the granules and the nucleoli are described as "highly distorted" and "very much shrunk." Furthermore, insects which took up water because of increased cuticular permeability are described, in contrast to the 1957 report, as having cells which "showed a very dense mass of stainable colloids in their cytoplasm." The secretory matter is in this paper interpreted as anti-diuretic in nature. These two reports appear to be conflicting.

The cytological observations in the present study agree with those of Pflugfelder (1937) who reported that the corpora cardiaca of desiccated phasmids showed a large accumulation of granules.

Certain evidence from several investigators seems to favor the view that the brain and retrocerebral complex produce an anti-diuretic hormone. Stutinsky (1953) delayed urine elimination in the rat with injection of extracts of the pars intercerebralis or of the corpora cardiaca and allata of *Blabera fusca*. Altmann's experiments (1956) with the honeybee and Raabe's work (1959) on phasmids support the existence of an anti-diuretic factor in the corpora cardiaca.

FIGURE 2. Type A neurosecretory cells from those of normal and dehydrated animals. A, pars intercerebralis of normal animal (Group A); B, pars intercerebralis of dehydrated animal (Group D); C, thoracic ganglion of normal animal (Group A); D, thoracic ganglion of dehydrated animal (Group D). Dehydrated animals show a great accumulation of granules in the cells of the pars intercerebralis and in the thoracic ganglia the cells have clumps of granules (G) adjacent to the trachea (T).
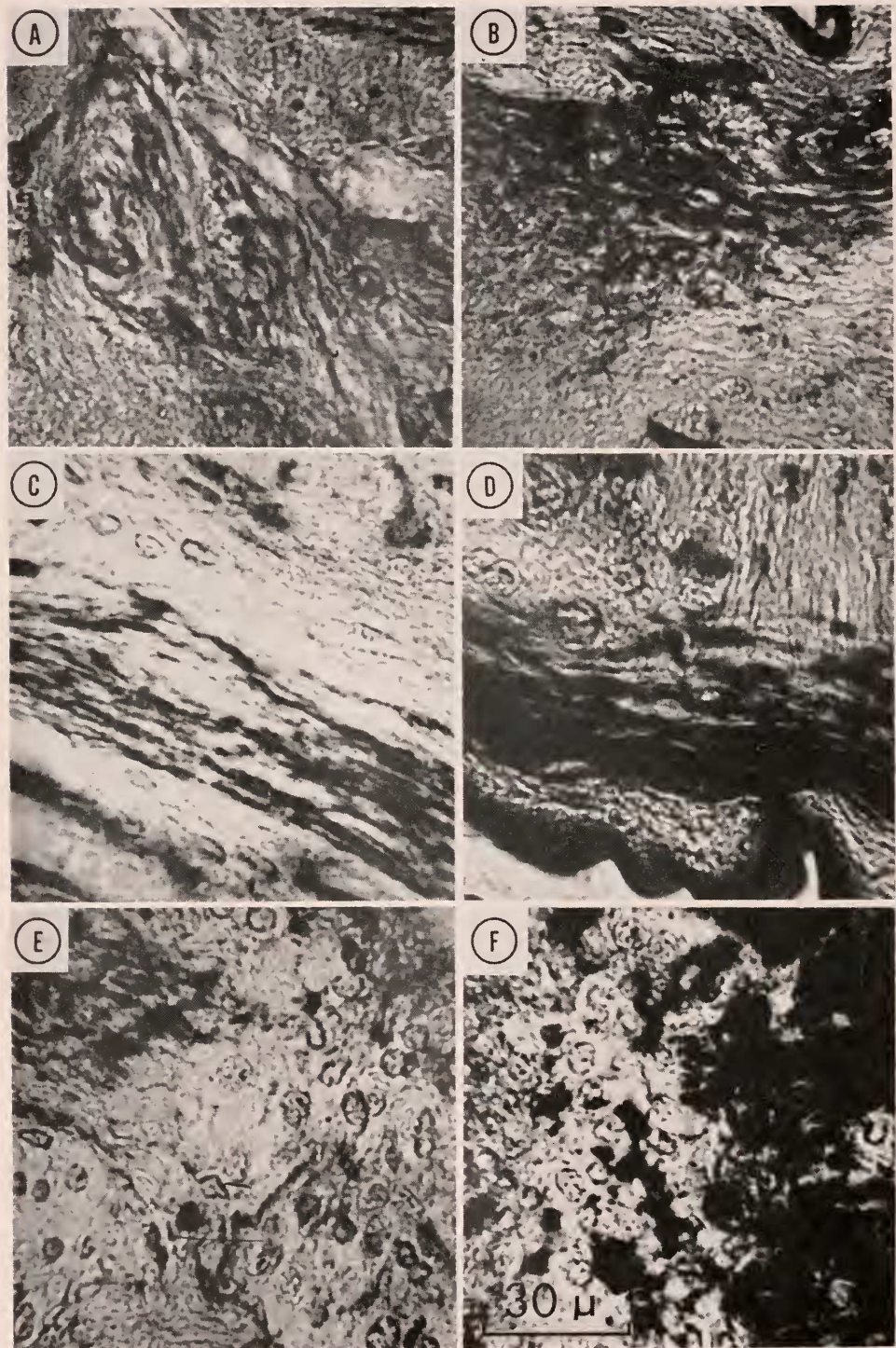
FIGURE 3.

However, other evidence indicates a diuretic principle in the retrocerebral complex. Nuñez (1956) interprets his results with a coleopteran (*Anisotarsus*) as indicating a diuretic principle in the dorsal part of the brain and corpus cardiacum, based on the fact that if these parts are excised the abdomen swells with increased fluid. Day (1943) has observed polydipsia and bloating in a few flies after removal of the corpus allatum. Dethier and Evans (1961) have pointed out, however, that allatectomy could have caused injury to the recurrent nerve. They show that cutting this nerve causes polydipsia and bloating in flies, but were not able to produce these effects as a result of allatectomy. Removal of the medial neurosecretory cells in a few cases caused bloating, but this is not interpreted as evidence for a hormonal mechanism since these cells are also in neural contact with the recurrent nerve. However, Altmann (1956) found that extracts of the corpora allata increase water uptake by honeybees and also increase the excretory rate of Malpighian tubules.

Emerging from this confused assortment of information is the general impression that the brain and retrocerebral complex probably produce both anti-diuretic and diuretic factors, which may or may not be confined to specific cell groups or endocrine structures. In all probability there are species differences that contribute to some of the confusion.

As for the observations regarding changes in the A cells of the thoracic ganglia, little can be said in the way of interpretation except that they may be involved with osmoregulation. There are no reports in the literature known to the authors which relate osmotic regulation to the thoracic ganglia. No explanation for the peculiar clumping pattern can presently be offered, but since the site of clumping is always associated with a trachea some relationship to gas-diffusion gradients may be indicated. The fact that cytological responses to states of hydration are observed in the brain, and water-regulating hormones must surely be there, argues for the probability that the thoracic ganglia similarly will be found to be sources of such hormones. Current studies on other hormonal systems of the cockroach (Ralph, 1962), showing the widespread segmental distribution of hormones, provide strong evidence, by analogy, that certainly some of the ventral cord ganglia are involved with water-regulation hormones.

The interesting results of Nuñez (1956) on abdominal cord sectioning could actually be interpreted to mean that abdominal ganglia are sources of diuretic hormone, which fails to be released when severed from the central nervous system. (We found no cytological evidence for such a role, however.) Nuñez interprets the abdominal ganglia instead as sites of sensing devices which respond to swelling of the abdomen upon intake of fluid and trigger release of diuretic hormone from the brain. The concepts of Dethier and Evans (1961), most interestingly, appear to place these mechanoreceptors in the head vicinity and, furthermore, they suggest a strictly neural regulation of water intake.

FIGURE 3. Corpora cardiaca and fiber tracts between the pars intercerebralis and corpus cardiacum of normal and dehydrated animals. A, transverse section of tracts in normal animal (Group A); B, transverse section of tracts in dehydrated animal (Group D); C, longitudinal section of tracts in normal animal (Group A); D, longitudinal section of tracts in dehydrated animal (Group D); E, corpus cardiacum of normal animal (Group A); F, corpus cardiacum of dehydrated animal (Group D). Greater amounts of granular material are seen in the dehydrated animals.

The literature, obviously then, provides a confusing background for attempting to interpret the cytological findings of this investigation. Appropriate assays of all neurosecretory structures will be required before it can be concluded how many water-regulating hormones there are, in what neural structures they are found, and what their actions may be. Work in this laboratory is currently proceeding along lines that may help clarify some of these aspects.

Summary

Regulation of water content in the cockroach, *Blaberus giganteus,* may be influenced by the secretions of specific neurosecretory cells. Under conditions causing dehydration (a) the type A cells of the pars intercerebralis show greater than normal amounts of stainable granules, and (b) the type A cells of the thoracic ganglia show clumping of granules toward the surface adjacent to a trachea and have a coagulated-appearing cytoplasm. Salt injections of $4 \times$ NaCl in Hoyle's solution produced the same cytological appearances as dehydration. Specific cytological changes were not seen in any other neurosecretory cells.

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